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Quality Assessment of a Traditional Unani Medicine: Kushta-e-Gaodanti

Md. Yaqub Khan*, Poonam Gupta, Bipin Bihari, Aparna Misra

Saroj Institute of Technology & Management, Ahimamau P.O. Arjunganj Sultanpur Road,
Luck now- 226002

*Corresponding Author E-mail: khanishaan16@yahoo.com

Kushta-e-gaodanti was prepared as per the method mentioned in Unani formulary. The raw materials, intermediates obtained during the preparation of kushta and the final product were characterized using modern analytical techniques like Fourier transform infrared spectroscopy (FTIR). X-ray powder diffraction (XRPD) and thermo gravimetric analysis (TGA). The study shows that the mineral Gaodanti (calcium sulphate dihydrate) is converted into calcium sulphate hemi hydrate on first calcination in earthen pot sealed with the process gil-e-hikmat. Further on calcination this intermediate is transformed to kushta-e-gaodanti which is orthorhombic α -calcium sulphate anhydride. Kushta-e-gaodanti is a fine powder with particle size 7-8 micrometer, calcium content 29.32%, bulk density 0.928, tapped density 1.268, angle of repose 36.67° and the value of Carr's index is 47.08. Loss on drying at 110°C and loss on ignition was not more than 0.5 %w/w and 0.05% w/w respectively. Microbial load of the preparation was found negative for the presence of *Escherichia coli*, *Salmonella species* and *Staphylococcus aureus*. Total aerobic count was under acceptance limit. Trace element analysis of bhasma by ICP-OES revealed the presence of some other important metals like arsenic, lead, chromium, cadmium, mercury, tin under acceptable limits at prescribed dose.

Keywords - FTIR; calcination; Unani traditional medicine

Introduction

'Unani' means medicines which are a symbol of life. The name is derived from the word 'Ionian' which originated in Greece. 'Tibb' means the knowledge of the states of the human body in health and decline of health. 'Tibb-E-Unani', is hence an age old, time tested system of medicine dating back 5000 years to Greece. Its popularity outside India is mainly in Arabian and Muslim world. In this system of medicine, drugs of herbal, animal and metal origin are used for cure of diseases. Kushta is an important class of unani medicinal preparation obtained by calcinations of metal, mineral and animal drugs. Kushta being metal preparation are inorganic in nature. Kushtas of iron, calcium, copper, tin silver, gold, lead and zinc are commonly used. Kushta containing calcium differs from each other in their medicinal properties depending on the source of calcium used during synthesis. Kushta-e-gaodanti is a calcium containing preparation popularly used for the treatment of mahallil-e-waram (inflammation), hammiyat (pyrexia), tashannuj (cramp), falij (haemiplegia), waj-ul-mafasil (rheumatism), nigras (gout) and

malaria in the dose range of 200 to 500 mg/Day. It is prepared using gypsum (gaodanti) as source of calcium.

The general procedures for synthesis of kushta drugs begins with the process of purification [ghasl-e-adviyah], cleaning [tasfiyah] and detoxification [tadbir-e-adviyah]. Further drugs are finely grounded in pestle and mortar with specified juice of known drug for given time. Then mixture is sealed in an earthen pot using special process and calcinated in closed crucible in pits of different sizes having varying number of cowdung cakes. This provides different intensity of heat. The process is repeated till the kushta is obtained. The tests and observations to monitor quality of kushta described in the unani text are highly subjective. Use of modern analytical techniques will help in establishment of quality control methods for traditional preparations. Previous studies of this lab have also reported the use of modern analytical techniques in quality assessment of kushta or bhasma formulation. In the present work modern analytical techniques like FTIR and XRD and TGA are used for rapid characterization of raw material, intermediate and final product. Further attempt has been made to evaluate kushta for some physical, chemical, microbial parameters. Accumulated toxicity data on the hazardous effects of heavy metals have made health scientists afraid of heavy metals. This is because if heavy metals are present in higher concentration than permissible they can also exert toxic effect. As a result, a through investigation is needed for the kushta with regards to their elemental composition: [1]

Table1. Evaluation of kushta-e-gaodanti. [2]

Parameters	Test	Inference
Identity	Macroscopic	Non lustrous, grayish white, fine powder
	Physical Properties	Bulk density - 0.928
		Tapped density- 1.268
		Angle of response- 36.67°
		Carr's index- 47.08
		Loss on drying at 110°C- <0.5 % w/w
		Loss on ignition- <0.05% w/w
Purity	FTIR	Calcium Sulphate anhydrite
	Contaminating fungus (Yeast and mould)	<1 * 10 ⁴ CFU/g
	Total Aerobic Count	<1 * 10 ⁵ CFU/g
	<i>Escherichia Coli</i>	Absent
	<i>Salmonella spp.</i>	Absent
	<i>Staphylococcus aureus</i>	<1 * 10 ² CFU/g
	Arsenic	<0.14 µg/kg body weight/day
	Cadmium	<0.09 µg/kg. body weight/day
	Lead	<0.29 µg/kg. body weight/day
	Total mercury	<0.29 µg/kg. body weight/day
Quantity	Quantitative Test	Calcium content: 29.32%

Materials and Methods:

Materials

Materials were procured and authenticated by the Tayyebi dawakhana unani (Indore) pvt. Ltd. Transparent plates like crystal of Gaodanti [gypsum] were selected for synthesis of kushta. Joshanda-e-asgand nagori is decoction obtained from roots of *Withania somnifera*. The decoction was filtered using muslin cloth.^[3]

Preparation of Kushta-e-gaodanti

Small pieces of gaodanti (gypsum) were first cleaned with hot water. Cleaned sheets of gypsum were grounded for eight hours with equal proportion of decoction of Joshanda-e-asgand nagori, dried and pressed to form small cakes. The cakes were kept and sealed in earthen disk by gil-e-hikmat process. Here two earthen disks are kept opposite; cakes are placed between them and sealed using soil paste. This apparatus is then dried and kept in pits. Pit is filled half with 25 kg of cowdung cake, ignited and then cooled. Drug cakes obtained after first calcinations [QC K 104] is taken out from the apparatus; powdered, treated with Joshanda-e-asgand nagori and triturated for eight hours. The mixture was pressed in form of cake and dried. The cakes were calcinated as above to obtain the second intermediate [QC K 108]. The procedure was repeated two more times till the sample showed the entire traditional test for kushta positive, to obtain the final product [QC K114]. Three batches of kushta were prepared and approved by two experts of Unani formulation unaware of the procedure. Kushta used for evaluation is a mixture of all the three batches of kushta prepared.

Physical evaluations

Loss on drying, loss on ignition, bulk density and tapped density were determined using standard pharmacopoeial Methods. Values of bulk density and tapped density were used to indirectly calculate flowability by deriving % compressibility (Carr's index). Static angle of repose was determined by funnel method. Particle size distribution studies were carried out using particle size analyzer (Sympatec, Germany). The kushta was added to distilled water and sonicated for 30 seconds and analyzed.

Chemical evaluations

Fourier transforms infra-red spectroscopic studies:

The raw material, intermediates obtained after each calcinations process and final product were scanned using thermonicolet IR-200 spectrophotometer with DTGS detector in the region of 650 to 3600 cm^{-1} . Each spectra is an average of 24 scans of 2 cm^{-1} resolution. Sampling was done using attenuated total reflectance (ATR) assembly with a sample holder of Zn-Se crystal.

X-ray powder diffraction analysis [XRPD analysis]:

All the samples were scanned on Phillips make X-pert powder diffractometer and 2 θ scan was from 10° to 100° using Ni filter Cu K alpha radiation and NaI scintillator.

Thermo gravimetric Studies:

The thermo gravimetric analysis was done on Perkin Elmer series TG analyzer. The thermogram were recorded from 40°C to 1000°C at the heating rate of 10°C per minute in air atmosphere.

Table 2. Observed Infra red fundamental modes [cm⁻¹] of gaodanti, Intermediates and kushta-e-gaodanti recorded at room temperature. [4, 5]

Sr No.	Present study			Reference			Assignment
	Godanti [QC K 101cm ⁻¹]	Intermediate [QC K 101 cm ⁻¹]	Kushta-e-gaodanti [QC K 114 cm ⁻¹]	Calcium Sulphate Dihydrate [cm ⁻¹]	Calcium Sulphate Hemihydrate [cm ⁻¹]	Calcium Sulphate Anhydride [cm ⁻¹]	
1.	669	658	674	667,668	658	676	$\nu_4 \text{SO}_4$
2.	1005	1007	1014	1004	1008	1014	$\nu_1 \text{SO}_4$
3.	-	1096	1096	-	1096	-	$\nu_3 \text{SO}_4$
4.	1117	1113	1128	1120	1116	1124	$\nu_3 \text{SO}_4$
5.	1141	1153	-	1145	1153	1135	$\nu_3 \text{SO}_4$
6.	-	-	1155	1155	1168	1155	$\nu_4 \text{SO}_4$
7.	1621	1622	-	1621	1623	-	$\nu_2 \text{H}_2\text{O}$
8.	1685	-	-	1685	-	-	$\nu_2 \text{H}_2\text{O}$
9.	3405	3409	-	3405	3550	-	$\nu_1 \text{H}_2\text{O}$
10.	3499	3500	-	3495	3560	-	$\nu_1 \text{H}_2\text{O}$
11.	3555	3616	-	3555	3615	-	$\nu_3 \text{H}_2\text{O}$

Table3. X-Ray powder diffraction analysis of gaodanti and kushta-e-gaodanti. [6]

XRPD Analysis	Crystal System	Axis Ratios	Unit cell Parameters	d-Spacing [Å]
Godanti [Calcium Sulphate Dihydrate]	Monoclinic Prismatic	a:b:c=0.3747:1:0.4143	a= 5.68 Å b= 15.18 Å c= 6.29 Å	7.63 [85%] 4.22 [22%] 3.77 [100%] 2.67 [43%]
Kushta-e-gaodanti [Calcium Sulphate Anhydride]	Orthorhombic	a:b:c=0.9992:1:0.8916	a=6.993 Å b=6.995 Å c=6.245 Å	3.49 [100%] 2.849 [14 %] 2.328 [15%]

Determination of % calcium content:

Both raw material and final product were analyzed for calcium content. For the determination, the sample (0.1gm) was dissolved in 3 ml of dilute HCl and further diluted with 10ml of distilled water. The mixture was boiled for 10minutes, cooled and diluted to 50ml with distilled water. The mixture was then back titrated with 0.05M disodium edetate using eriochrome black as indicator. [7]

Inductive couple plasma analysis:

A Perkin Elmer Elan 6000 ICP-OES equipped with an As-91 auto sampler was used. Instrument was calibrated using reference standards of 1ppm and 10ppm. Approximately 0.1 gram of sample was accurately weighed into a metal free container and dissolved in 1ml of Aquaregia and heated on a hot plate to extract the metal. Then solution was filtered in a volumetric flask and washing of deionized water was added to it and volume made up to 10ml. The solution was used for analysis.

Microbial evaluations (Anonymous 1996):

Test for Contaminating fungus (yeast and mould):

10 g of the kushta was suspended in 100 ml of pH 7.2 phosphate buffer. 1 ml of the preparation was added to 15 ml of the liquefied potato dextrose agar medium in two petridishes at not more than 45°C and incubated at 25°C for 7 days. The dishes were observed and numbers of colonies were counted.

Total Aerobic microbial count:

10 g of the kushta was suspended in 100 ml of buffered sodium chloride-peptone solution pH 7. 0.1% w/v of polysorbate 80 was added to assist the suspension of poorly wettable substances. 1 ml of the preparation and about 15 ml of the liquefied casein soyabean digest agar was added to two petridish at not more than 45°C and incubated at 30°C to 35°C for 4 days. The dishes were observed and numbers of colonies were counted.

Test for *Escherichia coli*:

10 g of the kushta was suspended in 100 ml of buffered lactose broth by shaking in a sterile screw-capped jar. 0.1% w/v of polysorbate 80 was added to assist the suspension of poorly wettable substances. 1 ml of the preparation was transferred in a sterile screw-capped container and 50 ml of nutrient broth was added. Preparation was then shaken and allowed to stand for 1 hour and shaken again. The cap was loosened and jar was incubated at 37°C for 24 hours. The dishes were tested for presence of acid and gas as per standard procedure.

Test for *Salmonella*:

1 g of the kushta was suspended by shaking with 100 ml of nutrient broth in a sterile screw-capped jar and allowed to stand for 4 hours and shaken again. The cap was loosened and jar was incubated at 35°C to 37°C for 24 hours. 1.0 ml of the enrichment culture was added to each of the two tubes containing, 10 ml of selenite F broth and tetrathionate bile-brilliant green broth Respectively and incubated at 36°C to 38°C for 48 hours. Each of these two cultures, were subcultured on bismuth sulphite agar and brilliant green agar. Plates were incubated at 36°C to 38°C for 18 to 24 hours and observed for the presence of black-green or pink colony respectively.

Test for *Staphylococcus aureus*:

1 g of the kushta was suspended by shaking with 100 ml of nutrient broth in a sterile screw-capped jar and allowed to stand for 4 hours and shaken again. The cap was loosened and jar was incubated at 35°C to 37°C for 24 hours. 1.0 ml of the enrichment culture was added to soyabean-casein digest medium. Medium was examined for the presence of growth. A portion of medium was streaked on the surface of Vogel-Johnson agar and Mannitol-salt agar medium. Plates were incubated at 36°C to 38°C for 18 to 24 hours and observed for the presence of black and yellow colonies surrounded with yellow zones. [8,9]

Results:

Kushta-e-gaodanti prepared by traditional method as given in unani formulary is a fine grayish white powder. The results of evaluation of kushta-e-gaodanti are as shown in table 1. The IR spectra of the raw material and final product in region 650 cm⁻¹ to 4000 cm⁻¹. The peaks for

fundamental modes for sulphate and water molecules for gaodanti and kushta-e-gaodanti are identified. These IR peaks position are listed and compared in table 2.

Further 20% weight loss above 100°C observed in thermogravimetric analysis corresponds to presence of two molecules of water in gaodanti. The calcium content of kushta was found to be 29.32%. The results of ICP analysis revealed that metals like arsenic, lead, chromium, cadmium, mercury, tin under acceptable limit at prescribed dose^[10]

Microbial load of the preparation was found negative for the presence of *Escherichia coli*, *Salmonella* specie and *Staphylococcus aureus*. Total aerobic count was under acceptance limit.

Discussion:

The observed physical properties clearly show the poor flow property of kushta-e-gaodanti. The flow property can be improved by granulation of kushta. The FTIR studies of gaodanti shows ν_1 [SO₄] mode at 1005 cm⁻¹, ν_4 [SO₄] mode at 669 cm⁻¹ and ν_3 [SO₄] mode 1117 cm⁻¹ and 1141 cm⁻¹ respectively. The bending modes of water [ν_2] were observed at 1621 cm⁻¹ and 1685 cm⁻¹. Two stretching modes of water group [ν_3 and ν_1] were found at 3405 cm⁻¹ and 3555 cm⁻¹. These observations are similar to the reported one. This establishes its identity as calcium sulphate dihydrate. The pattern support the IR results, confirming raw material gaodanti [QC 103] as single phase monoclinic calcium sulphate dihydrate crystal having sharp and strong peaks with d spacing values at 7.49 Å, 4.25 Å, 3.77 Å and 3.05 Å and the lattice parameter as listed in table 3.

FTIR data of intermediate [QC K 104, QC K 108] as in table 2, shows ν_1 [SO₄] at 1007 cm⁻¹, ν_3 [SO₄] mode at 1096 cm⁻¹, 1113 cm⁻¹ and 1153 cm⁻¹, ν_4 [SO₄] mode at 658 cm⁻¹ and only one bending mode of water ν_2 at 1622 cm⁻¹. Further displacement occurs in the stretching modes of water to the region of 3550 cm⁻¹ to 3750 cm⁻¹ which reveals dehydration of gaodanti to calcium sulphate hemi hydrate on calcination.

The kushta-e-gaodanti [QC K 114] shows ν_1 [SO₄] sulphate mode at 1014 cm⁻¹, ν_4 [SO₄] mode at 674 cm⁻¹ but stretching and bending modes for water molecule are absent. The d spacing values 3.49 Å, 2.84 Å, 2.32 Å, 2.20 Å and lattice parameter as in table 3 obtained by XRPD analysis indicates complete dehydration of gaodanti to orthorhombic α - calcium sulphate anhydride insoluble in water. TG investigation of kushta-e-gaodanti shows no significant weight loss affirming the above results.

The analysis of the result shows that the overall process of formation of kushta-e-gaodanti involves gradual dehydration of gaodanti (monoclinic calcium sulphate dihydrate) to orthorhombic α -calcium sulphate anhydride. This transformation occurs via formation of calcium sulphate hemi hydrate as intermediate. The characterization techniques like FTIR, XRPD, TGA, PSA which have been used in the present studies can be used as quality control methods for characterization of samples in industry not only to check uniformity of the samples marketed by manufacturer but also to ensure that each step is been followed and product marketed are kushta and not the intermediate. A routine use of such scientific techniques will lead to standardization of the product to a certain extent and would definitely help in building confidence in use of such products for medication.^[11, 12]

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